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Effect of acetyl-L-carnitine on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity

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Abstract—1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is believed to induce neurotoxicity by inhibiting mitochondrial oxidative metabolism, whereas acetyl-L-carnitine (ALC) facilitates this process by transporting fatty acids into mitochondria for β oxidation. We investigated whether large doses of ALC given by gavage for 1 week before and 1 week after MPTP could ameliorate MPTP neurotoxicity in mice. We found that ALC had no effect on MPTP-induced depletion of striatal dopamine and its metabolites.

L-Carnitine and acylcarnitines, including acetyl-L-carnitine (ALC*), play an obligatory role in the β-oxidation of fatty acids by mediating the translocation of acyl groups, CoA and acetyl-CoA, across the mitochondrial inner membrane [1, 2]. On the other hand, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes acute parkinsonism in humans [3, 4], and produces in experimental animals a syndrome that is clinically, pathologically, biochemically and pharmacologically similar to Parkinson disease [5]. MPTP is believed to induce selective toxicity at central dopaminergic neurons via the end product of its oxidation, 1-methyl-4-phenylpyridinium, which inhibits oxidative metabolism at complex I of the mitochondrial electron transport chain [6, 7]. Furthermore, recent studies suggest that Parkinson disease subjects may have a defect in complex I of mitochondria of platelets [8], muscle [9], and brain [10].

For these reasons and in view of a recent report suggesting that ALC protects against MPTP-induced parkinsonism in primates [11], we examined whether ALC protects against the MPTP-induced depletion of dopamine (DA) and its metabolites in mice striata. ALC was given orally in relatively large doses before and after MPTP administration to black mice, which are known to be susceptible to MPTP neurotoxicity [12]. A preliminary account of these results has been presented [13].

Materials and Methods

Adult male C57Bl mice (22–25 g) were used. ALC (supplied by Sigma Tau, Rome) was dissolved in water and administered by gavage in doses of 50 or 250 mg/kg, twice daily for the 2-week duration of the experiment. Control mice received water at the same schedule. One week after starting ALC, MPTP·HCl or vehicle was injected subcutaneously, 20 mg of MPTP base/kg, twice daily (6 hr apart) on 2 consecutive days, for a total MPTP dose of 80 mg/kg. At the end of the experiment (2 weeks after ALC and 1 week after MPTP), the mice were killed and their striata assayed for DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), by HPLC with electrochemical detection [14].

Results from the two striata of each mouse were averaged, and the averages were used for statistical analyses. Significant differences between the groups was assessed by ANOVA with Scheffe post-hoc analysis. Significance was considered at $P < 0.05$.

Results and Discussion

MPTP (80 mg/kg over 2 days) induced no gross behavioral effects in C57Bl mice, but it reduced striatal DA by 60–70% with a concomitant decrease in striatal DOPAC and HVA levels (Tables 1 and 2). This was consistent with our previous experience [12].

Mice that received ALC in doses of 100 or 500 mg/kg/day for 2 weeks did not appear to be different from control mice. Also, ALC did not alter striatal DA or its metabolites (Tables 1 and 2). More important, both doses of ALC were ineffective in ameliorating the MPTP-induced reductions in striatal DA and its metabolites (Tables 1 and 2). The experiment using the high dose of ALC was performed after failure of the lower dose to protect against MPTP toxicity. The ALC doses are arbitrary because of the lack of data on intestinal absorption, half-life and pharmacokinetics of ALC. However, the ALC doses that we used were much higher than the oral dose that increases longevity [15] and improves memory [16], or the intraperitoneal dose that increases γ-aminobutyric acid and glutathione levels in the substantia nigra [17] of rats.

Our results are not consistent with the report that ALC (20–50 mg/kg/day, intramuscularly) protected against MPTP-induced toxicity in monkeys, which was based mostly on clinical and electroretinographic observations [11], although we used much larger ALC doses and for extended periods before and after MPTP intoxication. We have no good explanation for the conflicting results other than the different species of experimental animals and the fact that we only assessed MPTP neurotoxicity by biochemical measurements of striatal DA and its metabolites.

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* Abbreviations: ALC, acetyl-L-carnitine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; and HVA, homovanillic acid.

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Table 1. Effects of acetyl-L-carnitine (100 mg/kg/day) on MPTP-induced depletion of striatal dopamine and its metabolites

Condition	Group	N	DA	DOPAC	HVA
No ALC No MPTP	1	4	11.73 ± 0.97	1.02 ± 0.06	1.55 ± 0.06
ALC No MPTP	2	5	12.34 ± 0.95	1.17 ± 0.15	1.42 ± 0.09
No ALC MPTP	3	5	3.40 ± 0.69	0.55 ± 0.09	1.11 ± 0.09
ALC MPTP	4	5	3.96 ± 0.57	0.39 ± 0.04	1.05 ± 0.05

Results, in μg/g wet weight of striatum, denote means of the number of mice (N) ± SEM. For DA, groups 1 and 2 were each significantly different from groups 3 and 4 at $P < 0.01$. For DOPAC, groups 1 and 2 were each different from group 4 at $P < 0.01$, group 1 was different from group 3 at $P < 0.01$, and group 2 was different from group 3 at $P < 0.05$. For HVA, group 1 was different from group 3 at $P < 0.05$ and from group 4 at $P < 0.01$, and group 2 was different from group 4 at $P < 0.05$. There were no significant differences between the results of groups 1 and 2 or between groups 3 and 4 (ANOVA with post-hoc Scheffe analysis).

Table 2. Effects of acetyl-L-carnitine (500 mg/kg/day) on MPTP-induced depletion of striatal dopamine and its metabolites

Condition	Group	N	DA	DOPAC	HVA
ALC No MPTP	1	7	10.54 ± 0.24	1.02 ± 0.07	0.95 ± 0.04
No ALC MPTP	2	7	4.13 ± 0.38	0.46 ± 0.03	0.62 ± 0.03
ALC MPTP	3	8	3.81 ± 0.37	0.51 ± 0.05	0.67 ± 0.05

Results, in μg/g wet weight of striatum, denote means of the number of mice (N) ± SEM. The results of group 1 were significantly different from groups 2 and 3 at $P < 0.01$. There were no significant differences between the results of groups 2 and 3 (ANOVA with post-hoc Scheffe analysis).

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